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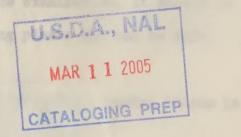


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PLANT NEMATOLOGY

LABORATORY MANUAL

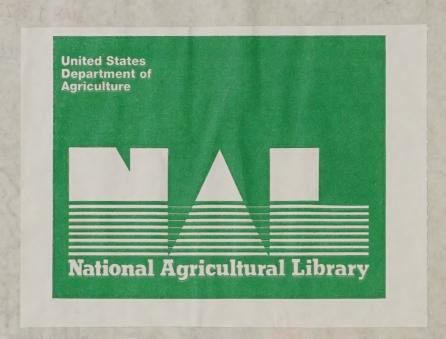
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Prepared by the Nematology Section U.S.D.A.

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Collecting Soil and Plant Samples

In collecting plant and soil samples for nematode examination, it should be kept in mind that the nematodes feed on roots of growing plants and can be most easily found where their food is abundant (Fig. 1).

A good way to proceed in sampling soil around the roots of growing plants is as follows:

- 1. Get a shovel, trowel, soil sampling tube or other digging implements.

 Also a supply of plastic (or paper) bags, and some labels.
- 2. Select the plant to sample. The best plants to sample are those which are not yet seriously damaged and still have plenty of living roots. Plants with mostly dead roots will not have many plant parasitic nematodes because there is little food for them.
- 3. Dig samples to include living roots and some of the adjacent soil. If the plants are small, dig up a whole root system. If the plant is larger, take only part of the root system. A sample of about 500 cc is sufficient.
 - 4. Place the sample in the bag and label it.
- 5. Protect samples from drying or from excessive heat. Either may kill many of the nematodes.
- 6. Extract the nematodes from the samples as soon as possible, using the methods described below.
- 7. If the nematodes are in the plant tissue, such as roots, stems or bulbs, collect plants which show the symptoms and keep from drying until the nematodes are extracted.

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 - 7. If the nematodes are in the plant tissue, such as roots, stems or bulbs, collect plants which show the symptoms and keep from drying until the nematodes are extracted.

Isolations of Nematodes from Soil Samples

Nematodes can be isolated from soil samples by: (1) the Cobb sieving and gravity method and (2) the Baermann funnel.

Sieving and Gravity Method for Soil

- 1. Put a soil sample of about 300 cc of soil in a bucket (Fig. 2A).
- 2. Add about 2 liters of water.
- 3. Mix the soil and water by stirring with a stick, then allow mixture to stand for 30 seconds.
 - 4. Pour the water through a 20-mesh sieve into a second bucket (Fig. 2B).
 - 5. Discard the debris on the 20-mesh sieve.
- Note: A larger proportion of the nematodes can be obtained by repeating 2 to 5 once or twice.
- 6. Discard the material in the first bucket. This consists of sand and heavy soil particles.
- 7. Pour the water in the second bucket back through a 60-mesh sieve into the first bucket (Fig. 3C).
 - 8. Wash the residue from the 60-mesh sieve into a beaker (Fig. 4E).
 - 9. Pour the water in the first bucket through a 200-mesh sieve.
 - 10. Wash the residue from the 200-mesh sieve into a beaker (Fig. 4E).
- the microscope. Cysts or adult females of the genus Heterodera will be found in the residue from the 60-mesh sieve. Cysts often float, so both the bottom of the dish and the surface of the water should be examined. Some of the larger nematodes will also be found in the residue from the 60-mesh sieve; the smaller nematodes will be found in the residue from the 60-mesh sieve; the smaller nematodes will be found in the residue from the 200-mesh sieve. Or: Place it in the Baermann funnel as described below.

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 - 9. Pour the water in the first bucket through a 200-mesh sieve.
 - 10. Wash the residue from the 200-mosh sieve into a beaker (Fig. hE).
- II. Four the material in the beskers into shallow dishes for examination by the microscope. Cysts or adult females of the genus Heteroders will be found in the residue from the 60-mesh sieve. Cysts often flost, so both the bottom of the dish and the surface of the water should be examined. Some of the larger nematodes will also be found in the residue from the 60-mesh sieve; the smaller nematodes will be found in the residue from the 200-mesh sieve. Or: Place it is the Baermann funnel as described below.

Note: Ordinary soil sieves, obtainable from scientific supply companies, can be used for extracting mematodes by the sieving method. The "20-mesh" sieve has 20 openings to the inch and each opening 0.840 mm. wide. The "60-mesh" sieve has openings 0.250 mm. wide and the openings in the "200-mesh" sieve are 0.074 mm. wide.

The Baermann Funnel

A Baermann funnel is made by attaching a short piece of rubber tubing to a funnel and placing a pinchcock on the tubing. The funnel is then placed in an upright position and partly filled with water (Fig. 4F).

- 1. Place the sample from which the nematodes are to be extracted in a beaker.
- 2. Place a piece of cloth over the beaker and fasten it with a rubber band.
- 3. Invert the beaker in the funnel so that the cloth is under the surface of the water.
 - 4. Leave the funnel undisturbed for 3 hours or longer.
- 5. Take a sample from the furnel by opening the pinchcock for a short time.

 The sample should not be more than 10 ml.
 - 6. Place the sample in a shallow dish for examination.

Uses of the Baermann Funnel

The Baermann funnel can be used for extraction of nematodes from (1) soil samples, (2) sieve residues obtained by sieving soil as described above and (3) plant material.

For Soil Samples

- 1. Mix the soil samples thoroughly.
- 2. Put about 50 cc in a beaker.
- 3. Put a cloth cover over the beaker and fasten it with a rubber band.
- 4. Invert the beaker in the Baermann funnel.

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 - i. Invert the besier in the Beemann funnel.

For Sieve Residues

- 1. Place a cloth over the beaker containing the sieve residue and fasten it with a rubber band.
 - 2. Invert the beaker in the Baermann funnel.

Plant Material

- 1. Cut the material into small pieces and place in a beaker.
- 2. Place a cloth over the beaker and fasten with a rubber band.
- 3. Invert the beaker in the funnel.

Notes

Extraction of nematodes by the Baermann funnel depends on active movements of the nematodes which work their way through the cloth. Dead nematodes will not be extracted by this method, so it cannot be used with preserved material. In addition to cloth, various kinds of paper have been used in the Baermann funnel. Paper towels work very well as do different kinds of thin porous paper.

Besides the method mentioned, there are numerous ways of supporting the cloth in the Baermann funnel. Wire rings, screen wire or clothes pins have been used.

Nematodes left too long in the Baermann funnel sometimes die from lack of oxygen.

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Microscopes and Accessories

The nematologist needs two microscopes as follows:

- 1. Dissecting Microscope with stand and mirror for substage illumination, equipped with oculars and objectives to give magnifications of about 6 to 10%, 20 to 30%, and 50 to 75%. The lowest magnifications (about 6 to 30%) are used for examination of plant samples for symptoms of nematode attack, and for dissecting plant tissue to isolate the contained nematodes. These magnifications are also used in the various steps of preparing slides. Using the higher magnifications, the skilled worker can make tentative identifications of some kinds of nematodes.
- 2. Compound microscope equipped with a condensor, three objectives and oculars to give magnifications of about 100X, 450X and 950X. The usual combination is objectives of 16 mm., 4 mm., and about 2 mm. focal lengths, and oculars of about 10X. The lowest magnification is used for locating nematodes on the slide, and for observation of the general size and shape of the body. With magnifications of about 450X, some details of internal and external structure can be seen sufficiently well to permit identification to genus and sometimes to species. The highest magnification, obtained with an oil immersion objective, is used for study of minute details and is necessary for identification of most species.

A light which can be used for either incident or transmitted illumination is needed for the dissecting microscope, and a good microscope lamp is needed for the compound microscope.

An eyepiece micrometer or filar micrometer is a useful accessory for the compound microscope.

A camera lucida is needed if drawings of nematodes are to be made.

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Finding Nematodes in Plant Tissue

Nematodes in plant tissue may be (1) active nematodes or (2) inactive.

Different methods are used in searching for each.

Active Nematodes

Active nematodes are able to move and are always slender. This kind of nematode can be found in plant tissue as follows:

- 1. Place a small piece of the plant tissue in a watch glass and cover with water (Fig. 5A).
- 2. Place the watch glass under the dissecting microscope, using a magnification of 10x to 30x.
 - 3. With dissecting needles, tear the tissue apart (Fig. 6B).
- 4. This frees the nematodes from the plant tissue and they can then be found in the water. Look for them in the bottom of the dish.
- 5. If no nematodes are found, set the dish aside for an hour or more. Then look again.

A very efficient method of finding active nematodes in roots is the "Incubation Technique" described by Young in 1954 (Fig. 7).

- 1. Wash the roots thoroughly.
- 2. Place the wet roots in a fruit jar with a cover.
- 3. Set the jar aside for several days.
- 4. Spray a small amount of water on the roots and then pour it off into a beaker.
 - 5. Examine this water under the dissecting microscope.

The Waring Blendor

When nematodes are numerous in plant tissue, they can be found as follows:

- 1. Place about 2 grams of washed plant material in a Waring Blendor and add about 100 ml. of water (Fig. 8A).
 - 2. Run the Blendor for 20 seconds.

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When memoiodes are numerous in plant tigme, they can be found as fellows:

- 3. Pour the material through a 60-mesh sieve over a 200-mesh sieve (Fig. 8B).
- 4. Wash the material on the sieves with a gentle stream of tap water.
- 5. Discard the residue on the 60-mesh sieve and look for nematodes in the residue on the 200-mesh sieve.
 - 6. Or: Place the residue from the 200-mesh sieve in the Baermann funnel.

Inactive Nematodes

Inactive nematodes are the species with "pear-shaped" or otherwise enlarged bodies, such as the root-knot nematodes. These will be partially or completely embedded in the plant tissue.

- 1. Place short pieces of the plant tissue in a watch glass under the dissecting microscope.
 - 2. Using dissecting needles, pull the tissue apart to expose the nematodes.
 - 3. Dissect the nematodes out of the plant tissue.

Note: Root-knot nematodes will be found mostly in roots with distinct knots, but are occasionally found in roots without distinct knots. The nematodes are in the knots, and their exact location is often marked by the presence of an egg mass. This is an irregular brown body about one millimeter in diameter on the surface of the root. It can easily be detached, revealing the nematode underneath.

Root-knot nematodes are more easily dissected out of roots which have been in 5% formalin for at least 24 hours than from fresh roots.

- 3. Pour the material through a 60-mesh sieve over a 200-mesh sieve (Fig. 8B).
 - i. Wach the material on the sleves with a gentle streem of tap water.
 - 5. Discard the residue on the 50-mesh sieve and look for nematodes in the
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Mounting Nematodes on Temporary Microscope Slides

Microscope slides of nematodes which will be usable for several weeks or months can be made as follows:

- 1. Place a drop of 5% formaldehyde in the center of a clean microscope slide (Fig. 10A).
- 2. Locate a nematode in the Syracuse Watch Glass with the dissecting microscope, using a magnification of about 15 to 30X.
- 3. Gently lift the nematode with the point of the bamboo splinter upward to the water surface. Keep it in focus under the microscope (Fig. 9).
- 4. When the nematode is just under the surface of the water, lift rapidly to bring it through the surface film.
- 5. Place the point of the bamboo splinter in the drop of 5% formaldehyde on the slide and withdraw it carefully. This will usually leave the nematodes in the drop (Fig. 10A).
- 6. Place the slide under the dissecting microscope and make sure that all the nematodes are at the bottom of the drop.
 - 7. Using forceps, carefully place a cover glass over the drop (Fig. 10B).
- 8. Still working under the microscope, use a small piece of filter paper, blotting paper or paper towel to absorb the excess liquid. Be careful that none of the nematodes are lost (Fig. 11A).
- 9. Seal the slide with either: (a) A mixture of one part of paraffin and one part of vaseline. This is kept hot and applied with a small brush. (b) ZUT, a special slide-sealing compound obtainable from Bennett's, 65 West First South Street, Salt Lake City 10, Utah. (c) Other materials including fingernail polish (Fig. 11B).
 - 10. Label the slide (Fig. 110).

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 - 6. Flace the slide under the dissecting microscope and make sure that all the nematodes are at the bottom of the drop.
 - 11.7. Using forceps, carefully place a cover glass over the drop (Fig. 103).
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 - 9. Seel the slide with either: (a) A mixture of one part of paraffin and one part of veseline. This is kept hot and applied with a small brush. (b) 207. a special slide-sealing compound obtainable from Bennett's, 65 West First South Street, Salt Lake City 10, Utah. (c) Other materials including fingernail polish (Fig. 118).
 - 10. Label the slide (Fig. 110).

Notes: A bamboo splinter suitable for picking up nematodes is made by splitting a piece of bamboo about 20 cm long to obtain a piece about 2 mm wide. is One end of this/sharpened with a knife to make a long and very fine point. The point is best made by trimming with a razor blade under the dissecting microscope. The finer the point, the easier it is to use (Fig. 9).

Cover glasses should be the thinnest obtainable, usually the "No. 1" or "No. 0" thickness. If cover glasses are too thick, the nematodes cannot be examined with the oil immersion lens. Diameter of cover glasses should be 12 to 18 mm.

The slide sealing mixture is painted on, working under the dissecting microscope to be sure that the edges of the cover slip are sealed.

Other Methods of Making Microscope Slides of Nematodes

- 1. Temporary slides for immediate use under the lower powers of the compound microscope can be made by simply placing the nematodes in a drop of water and then placing a cover glass. If the nematodes are moving, heat the slide very gently with a match. Try to heat just enough to stop movement.
- 2. Slides can also be made by mounting nematodes which have been preserved in 5% formaldehyde in lacto-phenol solution. (Melted phenol, 3 parts; lactic acid, 1 part; glycerine, 2 parts; water, 1 part.) Such slides deteriorate in a few months so that certain internal structures of the nematode are difficult to see. Cuticle details remain good. This is an excellent mounting medium for cysts of Heterodera species and for perineal patterns of Meloidogyne species.
- 3. Permanent slides are made by mounting nematodes in glycerine.

 See below under "Fixing and Preserving Specimens".

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Fixing and Preserving Specimens

The standard material for preserving nematodes or plants and soil containing nematodes is 5% formaldehyde. A good procedure is as follows:

- 1. Prepare 10% formaldehyde by proper dilution with water of commercial formaldehyde solutions containing about 36% to 40% formaldehyde.
- 2. Place the material to be fixed in water at room temperature or below and add an equal volume of 10% formaldehyde solution heated to just below the boiling point. This relaxes and kills the nematodes without danger of ruining them by overheating.
- 3. Store in tightly closed containers until ready for examination. Or, transfer to pure glycerine as described below.

Note: Nematodes, or plant material containing nematodes will remain in good condition for years in 5% formaldehyde. Most other materials which have been tried have given inferior results in the long run. Formalin can also be used cold and this is sometimes an advantage if cuticular structures are to be examined. If sufficient material is at hand, it may be desirable to divide it and use both hot and cold formaldehyde.

- 4. Prepare solution of 1.5% glycerine in 10% ethyl alcohol.
- 5. Place the nematodes in this solution in Bureau of Plant Industry Watch Glasses or other small shallow containers. (Note B.P.I. Watch Glasses are Syracuse watch glasses 27 mm. in diameter.)
- 6. Place the watch glass in a tightly closed container of about 200 ml. capacity with a 16 mm. by 57 mm. screw cap vial filled with desiccated calcium carbonate. The cap of the vial should have a hole about 2 mm. in diameter. The purpose of this procedure is to permit very gradual evaporation of the water and alcohol, leaving the nematodes in pure glycerine after 3 to 6 weeks. If evaporation is too rapid, the nematodes will often collapse.

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7. Transfer the watch glasses to a large desiccator containing calcium chloride until it is certain that all the water and alcohol have been evaporated. The nematodes can then be stored indefinitely in desiccated glycerine in tightly closed containers.

Making Permanent Slides

- 1. Keep a shallow dish of glycerin for use as the mounting medium and another dish containing short pieces of glass rod in the desiccator. The glass rods should have about the same diameter as nematodes, that is, should be of varying diameters in the range of 10 to 150 microns. These rods are obtained by cutting glass wool into lengths of several millimeters, or by drawing out threads from glass rods heated by a Bunsen burner.
- 2. Place a small drop of the mounting medium on a slide and transfer nematodes to it from the watch glasses.
- 3. Select three pieces of glass rod just a little smaller in diameter than the nematodes and place at the edges of the drops evenly spaced. These are to support the cover glass so that the nematodes are not flattened or distorted.
 - 4. Lower a cover glass gently over the drop of mounting fluid.
 - 5. Blot up excess glycerine from around the edges of the cover glass.
 - 6. Seal the slide with ZUT.

Note: Nematodes can be mounted on ordinary microscope slides, but there is a considerable advantage in the use of the metal slides invented by Cobb (1917). These consist of a piece of aluminum bent to hold a square cover glass and 2 pieces of cardboard. Nematodes are mounted between the square cover glass and a round cover glass and so can be examined from either side. The cardboard pieces are used as labels. Metal slides can be stacked and so stored in boxes without the slots which are needed for glass slides.

- 7. Transfer the watch girages to a large desirects containing calcium until it is cortain that all the water and alcohol have been evaporated.
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- Note Nemetodos can be nounted on ordinary microscope slides, but there is a mide blt adventage in the use of the metal slides invented by Cobb (1917).
 - here are nounted between the square cover glass and a round
 on star on be maine from either side. The cardboard pleces are
 eff for slass slades.

Nematode Anatomy

All nematodes at some stage of their life are elongate worms, the body shape ranging from nearly cylindrical with rounded ends to fusiform. Most nematodes are this shape all through their lives, but adult females of certain important plant parasites have enlarged bodies which are pear-shaped, lemon-shaped, kidney-shaped, or otherwise enlarged (Fig. 12). The only appendages nematodes have are short setae usually near the anterior end. Soil and plant parasitic nematodes are rather small, average length being about a millimeter, with a minimum length of about 0.25 mm. and a maximum of about 10 mm. The great majority of the species are less than 2 mm. long.

In the typical nematodes (Fig. 14) the mouth is terminal at the anterior end. is
There is a mouth cavity which/reduced to a stylet in plant parasitic forms.

Attached to the mouth cavity or stylet is the ocsophagus which leads to the intestine. The intestine leads to the rectum and ends at the anus, which is situated on the ventral side. The body from the anus to the posterior terminus is the tail.

In the female, the vulva is also situated on the ventral side and is a transverse slit. This leads to the ovaries which are tubes lying in the body cavity. There may be either one or two ovaries. At the posterior end of the male body are a pair of structures known as spicules. These normally lie in the body, but can be pushed out through the anus. They are used in copulation. Also leading to the anus of the male is a single tube in which sperms are formed called the testis. Rearely, there are two testes.

Nematodes also have an excretory system opening into a ventral excretory pore situated on the anterior part of the body, in most species. The excretory canal, leading to this pore is the only part of this system which can be seen.

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arterior part of the body, in most species. The excretory

There is also a nervous system, the only visible part of which is the nerve ring around the oesophagus, and this is usually difficult to see.

The body is lined with muscle fibers arranged in four sectors separated by two lateral chords, a dorsal and a ventral chord. These are usually difficult to see.

Sensory organs include papillae surrounding the mouth cavity, near the excretory pore and near the posterior end. At the anterior end are also two chemical sense organs called amphids. In the Phasmidia these are terminal and very small so are seldom seen. In certain of the Aphasmidia, the external amphids are conspicuous circles, spirals and similar forms.

The phasmids are generally located in the tail. These are two glands connecting with lateral pores located in the middle of the lateral fields.

Usually only the pores can be seen and often these are hard to locate. The canals leading to the surface can sometimes be seen in ventral or dorsal views.

The body of the nematode is covered with a transparent, colorless or slightly yellowish cuticle. This cuticle may be marked in various ways. Many nematodes have annules running at right angles to the body axis. These may be less than one mirron wide or as much as 5 microns wide. Often they are interrupted laterally by the "lateral fields" which are more or less elevated ridges running lengthwise of the nematode. In many Tylenchida, the lateral fields are divided into three or more strips by lines.

In one genus of the Tylenchidae (Criconema) the coarse annules are bordered by well developed rows of spines or scales.

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Nematode Systematics

Nematodes belong to the Phylum Nematoda according to some authors, and to the Phylum Aschelmintha according to others. It is generally agreed that they should be subdivided into 2 classes as suggested by Chitwood in 1933. These classes are (1) Phasmidia, and (2) Aphasmidia; that is, nematodes with phasmids and nematodes without phasmids.

The classes are divided into orders with names derived from that of the type genus and ending in "-ida" (example Rhabditida). Names of suborders end in "-ina" (Rhabditina); of superfamilies in "-oidea" (Rhabditoidea); of families in "idae" (Rhabditidae); and of subfamilies in "-inae" (Rhabditinae). The families are composed of genera and the genera of species. A few subspecies have been named.

About an equal number of species of soil, marine, freshwater, insect parasites and plant parasites are known. This may be only a tenth or less of the existing species. The nematode fauna of the soil and plants of the greater part of the world is unknown.

In this manual we are concerned principally with the plant parasitic nematodes. Since the plant nematologist often encounters free-living nematodes in his work, we will also discuss the most common genera of these.

The plant parasitic forms now known belong mostly to a single order, the Tylenchida, of the class Phasmidia. A few species of 3 genera of the superfamily Dorylaimoidea of the Aphasmidia are also plant parasites. Soil nematodes are found in both Phasmidia and Aphasmidia.

The best book on taxonomy of the soil and plant parasitic nematodes is
"Soil and Freshwater Nematodes", by T. Goodey. This was published in 1951 by
Methuen & Co., Ltd., London and by John Wiley & Sons, Inc., New York. It
contains description of the genera with which we are concerned and illustrations

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of a representative species of each genus. The next best book is MA Manual of Agricultural Helminthology*, by I. N. Filipjev and J. H. Schuurmans-Stekhoven, published by E. J. Brill, Leiden, Holland in 1941. This is obtainable from E. G. Stechert & Co., 31 East 10th Street, New York City.

With the keys in this manual and "Soil and Freshwater Nematodes", identification of the common soil and plant parasitic nematodes to genus is possible.

Nematode taxonomy is constantly growing and changing. New species of nematodes are constantly being found, and new genera are fairly common. In addition a certain amount of changing of species from one genus to another is constantly being done. Consequently, identification of nematodes to species is difficult and often nearly impossible unless the genus has been recently revised.

In using the keys, constant reference to the illustrations of this manual and to those of the reference books should be made. There is no substitute for illustrations.

el Heiminthology", by I. M. Wilipjev and J. H. Schummans-Stehboven, F. E. Erill ette Holland in 1961. This is obtainable from E.

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Identification of Nematodes

The attached keys are used for nematode identification. The "Key to the most Common Nematodes of Agricultural Soils and Plants" will facilitate identification to family or subfamily.

As the first step a decision is made as to whether the nematode belongs to the Phasmidia or Aphasmidia. This is best determined by the form of the oesophagus. In the Phasmidia we find the oesophagi like those shown in figures 15 and 16.

Note that the "Rhabditoid" oesophagus has a distinct bulb at the base and that the bulb contains a valve of distinctive shape. This valve is easy to see (Fig. 15A).

The "Diplogasteroid" oesophagus is of the same general shape but the valve is lacking (Fig. 15B).

The other 3 types of oesophagi, "Tylenchoid", "Neotylenchoid" and "Aphelenchoid" are characterized by a stylet which is always present and easy to see. Stylets may be variations of any of the forms shown in figure 17 A-E. Attached to the stylet is a thin oesophageal tube. In all except the Neotylenchoid oesophagus (Fig. 16C), this tube leads to an oval "Valve" in a "median oesophageal bulb", then continues on to the intestine.

Males of some species of the Phasmidia may have a distinct bursa as shown in figure 14 and in figure 19D. Mature females of some genera have enlarged bodies as shown in figure 12.

In the Aphasmidia, the most common types of oesophagi are the "Plectoid", "Cylindrical" and "Dorylaimoid" as shown in figure 17A-C.

Note that the "Plectoid" oesophagus has a basal bulb as in the Rhabditoid type, but that the valve is elongated (Fig. 17C).

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The athached keys are used for nemateds identification. The "Ney to the "Common Nematedes of Agricultural Soils and Flants" will facilitate identifica-

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Males of some species of the Phasmidis may have a distinct bures as thown n fi are li and in figure 19D. Mature females of some papers have enlarged bodies

In the Aphaemidis, the most common types of vesophagi are the "Flectoid", lylindrical" and "Donylaimoid" as shown in figure 17A-C.

Note that the "Pleateid" assophages has a basis balb as in the Rhabditoid

The cylindrical type of oesophagus is easy to recognize. It is seen most often in the genus Mononchus, where it is associated with a large mouth cavity containing a tooth as shown in figure 17A.

The "Dorylaimoid" type of oesophagus consists of a narrow anterior portion and a broader posterior portion as shown in figure 17B. This is often, though not always associated with stylets of the types shown in figure 18F-H.

If the nematode belongs to the Phasmidia and has a stylet it will be placed in the Tylenchida, as shown by Item 2 of the Key. If the stylet is absent, it belongs to the Rhabditida.

Nematodes belonging to the Tylenchida are separated into Tylenchoidea and Aphelenchoidea by the characters named in Item 3 of the Key. If the stylet has well developed knobs as shown in figure 18A-D, the nematode belongs to the Tylenchoidea. The oesophageal tube in this group usually has an abrupt bend just posterior to the stylet. At this point can be seen a short branch which is the opening of the dorsal oesophageal gland. If this is present and the stylet knobs are absent, the nematode should have a long tail as shown in figure 19E-F.

On the other hand, if the nematode has a large prominent oesophageal bulb and a stylet with very small knobs or no knobs, it belongs in the Aphelenchoidea (Fig. 16B).

Phasmidia without stylets are placed in the Diplogasteridae if there is no valve in the basal oesophageal bulb (Fig. 15B).

If there is a valve in the dorsal oesophageal bulb, and the stoma is cylindrical as shown in figure 15A, the nematode is placed in the Rhabditoidea. If the stoma is not cylindrical, the nematode is placed in the Cephalobidae.

Nematodes belonging to the Aphasmidia are placed in the plectinae if the oesophagus is as shown in figure 18C. Most nematodes of the group have a spinneret on the tail as shown in figure 19H.

The sylindric type of cesophages is easy to recognise. It is seen most ofte of the control of the second that a large month cavity of the control of the con

The "Doryladmoi i" type of ossephagus cons sts of a narrow anterior portion and broader posterior portion as shown in figure 178. This is often, though not always associated with stylets of the types shown in figure 188-H.

If the nematode belongs to the Phasmidis and has a stylet it will be placed in the Tylenchida, as shown by Item 2 of the Key. If the stylet is shaent, it belongs to the Rhabditida.

Mometodes belonging to the Tylenchida are separated into Tylencheides and dea by the characters named in Item 3 of the Key. If the stylet has well developed knobs as shown in Tigure 18A-D, the nematode belongs to the Tylenchoides. The coscophagesi tube in this group usually has an abrupt bend fout posterior to the stylet. At this point can be seen a short branch which is the opening of the dornal coscoplagesi gland. If this is present and the stylet branch to the storm of the dornal coscoplagesi gland. If this is present and the

On the other hand, if the nematode has a large prominent ossophageol bulb and a stylet with very small knobs or no knobs, it belongs in the Aphelencheidea (Fig. 16B).

Phasmidis without stylets are placed in the Diplogasteridae if there is no a the basel oscophageal built (Fig. 15H).

If there is a valve in the dermal escophageal mib, and the stemm is opiladrical as shown in figure 15A, the nematode is placed in the Nhabditeridae. If the stone is not cylindrical, the sematode is placed in the Jephalobidae.

Wenatodes belonging to the Aphaemidia are placed in the placetimes if the secophages is as shown in figure 18C. Not nematodes of the group have a

If the oesophagus is cylindrical, as shown in figure 17A, the nematode is placed in the Mononchidae.

If the oesophagus is dorylaimoid, as shown in figure 17B, the nematode is placed in the Dorylaimoidea.

To identify a nematode:

- 1. Study the oesophagus. Look at four or five specimens if possible.
- 2. Compare the oesophagus with figures 15, 16 and 17, and decide which type it is.
 - 3. Use the key to identify the nematode to superfamily or family.
- 4. Find the section relating to this group in "Soil and Freshwater Nematodes".
- 5. Compare your nematode with the illustrations in the book. This will usually result in an identification to genus.

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Key to the Most Common Nematodes of Agricultural Soils & Plants

Key to the Most Common Nematodes of Agricultural Soils & Plants
1. Oesophagus rhabditoid (with valve in basal bulb as in Fig. 15A),
diplogasteroid (without valve in basal bulb as in Fig. 15B),
tylenchoid (Fig. 16A &B), or aphelenchoid (Fig. 16C). If
tylenchoid or aphelenchoid, always with stylet. Males of some
genera have a distinct bursa (Fig. 19D). Phasmids always present
but most often difficult to locate. Mature females of some genera
have a much enlarged body
Oesophagus plectoid (Fig. 17C), cylindrical (Fig. 17A) or dorylaimoid
(Fig. 17B). Often with setae. Males without bursa. Always with
elongate, vermiform body Aphasmidia 6.
2. Stylet always present. Shapes and proportions of stylets vary
greatly with genera and species, but are usually recognizable
as variations of the forms shown in Fig. 17A-E. Attached to
the stylet is a thin oesophageal tube which may be straight or
coiled
Stylet absent. Anterior portion of oesophagus muscular (striated)
(Fig. 15A-B)
3. Stylet mostly with well-developed knobs. If stylet knobs are absent,
tail is long and thin (Fig. 19E &F). Dorsal oesophageal gland
orifice near base of stylet, or at most, not more than one stylet
length posterior to stylet knobs. The oesophageal tube often has
an abrupt bend at this point. (Fig. 16A &B)
Stylet knobs small or absent. Median oesophageal bulb occupying nearly
full width of body (Fig. 16B). Tail rounded or conical (Fig. 19A-B).
Dorsal oesophageal gland orifice in median bulb and difficult to locate.
Oesophageal tube without abrupt bends Aphelenchoidea

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. Casephages rhabd toid (with valve in bess built as in Mg. 154),
Luplogastoroid (without valve in basel bulb as in Nig. 158),
tylenoboid (fig. 164 MB), or aphelemenoid (Fig. 160). If
y schold or aphelencicis, always with stylet. Meles of some
genera wave a distinct bursa (Fig. 199). Frankla always present
errang emos lo seismen entere de lecare de some genera
as siblinanti
esophegus plectoid (Fig. 170), cylindrical (Fig. 174) or derylahmoid
(Fig. 178). Often with actse. Males without burss. Always with
Stylet always present. Shapes and proportions of stylets wary
greatly with genera and species, but are usually recognizable
as variations of the forms shown in Fig. 17A-E. Attached to
the stylet is a thin oesophageal tube which may be straight or
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Stylet mostly with well-developed knobs. If stylet knobs are absent,
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orifice near base of stylet, or at most, not more than one stylet
length posterior to stylet knobs. The cesophageal tube often has
as abrupt bend at this point. Ilg. Ida
plet knobs small or absent. Median oesophageal bulb occupying mearly
fall width of body (Fig. 16B). Tail rounded or conical (Fig. 194-B).
real cesophageal gland orifice in median built and difficult to locate.
Descriptions Lengt

4.	Oesophagus rhabditoid, with valve in basal bulb (Fig. 15A) 5.
0es	ophagus diplogasteroid, without valve in basal bulb (Fig. 15B).
	· · · · · · · Diplogasteridae
5.	Stoma cylindrical, usually much longer than wide (Fig. 15A).
	· · · · · · · · · · · · · · · · · · ·
Sto	ma not cylindrical, or if nearly so, about as long as wide. Lips
	of some genera have distinct projections ranging in shape from
	rounded to elaborately ornamented
6.	Oesophagus plectoid (with basal bulb, Fig. 17C). Tail tip with
	a small projection (spinneret) Fig. 19H Plectinae
0es	ophagus cylindroid (Fig. 17A) or dorylaimoid (Fig. 17B)
7.	Oesophagus cylindroid. Mouth cavity large, subglobular, usually
	with one or more large teeth (Fig. 17A) Mononchidae
0es	ophagus dorylaimoid (Fig. 17B). Stylet often as shown in Fig. 13C.
	Some with much longer stylet, Fig. 18F-H, others with a tooth Dorylaimoidea
Not	e: Known plant parasites are either Tylenchida, or species of the
	genera Kiphinema, Longidorus and Trichodorus of the Dorylaimoidea.

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projection (spinners) Fig 1911
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IDENTIFICATION OF PLANT PARASITIC NEMATODES *

Nematodes which are plant parasites always have a stylet which can be recognized as some variation of the stylet shapes shown in Figure 18. Nematodes which do not have stylets, or which have stylets which are not obvious variations of the shapes shown in Figure 18 are not plant parasites. Having determined that the stylet resembles those shown in Figure 18, the nematode can be identified to genus by use of the key.

In using the key, constant reference should be made to the illustrations.

When a tentative identification has been made, the nematode should be compared with the illustrations in "Soil and Freshwater Nematodes", by T. Goodey (published in 1951 by Methuen & Co., Ltd., London, and John Wiley & Sons, Inc., New York).

This book illustrates only one species of each genus but other species of the genus will be very similar in most respects.

If a stylet is present, it is easily visible on good specimens at magnification of about 400 times. It is always good practice to examine several specimens; characters may be difficult to see on one specimen and easy to see on another. It should also be noted that the key can be used only for the identification of mature females. This is the stage most frequently encountered; for some of the species males are very rare.

^{*} As used here the term "plant parasites" includes parasites of fungi. It should be kept in mind that many kinds of nematodes probably feed only on fungi, others may feed either on fungi or higher plants. Probably most of the Neotylenchidae feed only on fungi, as do most species of Aphelenchus Certain species of Aphelenchoides and Ditylenchus are known to feed either on fungi or higher plants, and the same may be true for species of other genera of the Tylenchida as well. Thus, nematodes with stylets as shown in Figure 18 may or may not be parasites of higher plants.

In asing the key, constant reference should be made to the illustrations.

The illustrations in "Soil and Freshwater Mematodes" by T. Goodey (pablished with the illustrations in "Soil and Freshwater Mematodes" by T. Goodey (pablished 195 by Methman & Co., Ltd., London, and John Wiley & Sons, Inc., New York).

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of species of Aphelenchus Certain ranks of
are known to feed either on fungi or higher
for species of other genera at the Tylenem is
the stylets as shown in Figure 18 may or may for

	Key to the Mature Females of the Common Plant Parasitic Nematodes
1.	With median oesophageal bulb (Figs. 16A & C)
	Without median oesophageal bulb as in figure 16B; Or without median
	oesophageal bulb and with stylet as in Fig. 18F, G or H
2.	Stylet short (Fig. 16B) Neotylenchidae
	Stylet long (Fig. 18F-H)
3.	Stylet straight without enlargement at base (Fig. 18F)Longidorus
	Stylet with enlargement at base
4.	Stylet straight with oval enlargements at base (Fig. 18G). Xiphinema
	Stylet curved (Fig. 18H)
5.	Body of mature female pear-shaped, lemon-shaped or enlarged and saccate.
	(Fig. 12A-G.) Found in roots of plants, either embedded or attached
	by neck, some as cysts in soil
	Body of mature female much longer than wide (Fig. 12 H-L)
6.	Body of mature female pear-or lemon-shaped (Fig. 12A-C)
	Body of mature female saccate (Fig. 12D-G)
7.	Body of mature female pear-shaped, white, (Fig. 12A). Found completely
	embedded in roots, nearly always in distinct knots Meloidogyne
	Body of mature female pear-shaped or lemon-shaped, (Fig. 12B-C) white,
	yellowish, or brown according to age, attached to root by neck only,
	or found as a brown cyst in soil Heterodera
8.	Mature female embedded in plant root, often in knot, body shape ovoid to
	spheroid with elongated "tail", vulva nearly terminal.
	(Fig. 12F-G) Nacobbus
	Mature females attached to root by neck, body more or less kidney-shaped.
	(Fig. 12D-E)
9.	Vulva at 90% of body length, (Fig. 12E) parasites of citrus and clives
	Vulva at 72% of body length, parasites of numerous plants, mostly annuals.
	(Fig. 12D) Rotylenchulus

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eshincaelviceM
- long (Fig. P-H)
prichtmoi. (W. 181 .g.M) ered is immersion front by idgisate office
Stylet with enlargement at base
braight with oval enlargements at base (Fig. 186). Maphinuma
Stylet surved (Fig. 18H)
Budy of me bure female pear-shaped, lemon-shaped or enlarged and secosds.
(Pig. 124-6.) Found in roots of plants, either embedded or sitsched
. do e e e e e e e e e e e e e e e e e e
Body of mature female much longer than wide (Fig. 12 H-L)
Body of matur !emale pear-or lemon-shaped (Fig. 12A-C)
ody of mature female saccate (Fig. 121
of mature female pear-shaped, white, (Fig. 12A). Found completely
embedded in roots, nearly slways in distinct knot lot
Body of mature female peer-shaped or Lemon-shaped, (Fig. 12B-C) white, '
yellow, or brown according to age, attached to root by neck only,
or found as a brown eyet in soil
temals embedded in plant root, often in knot sody shape evoid to
females attached to root by neek, body more or less iddney-shaped.
Q
Walve at bod length, (Fig. 12H) particles of citrus and clives
the state of the latest and the late

10.	Tail long and thin, more than 6 times anal body diameter (Fig. 19E,F) 11
	Tail not long and thin, but rounded, or conical and more or less pointed
	(Fig. 19, A, B, C, G, H, I)
11.	Tail tip frequently clavate, (Fig. 19F), one or two ovaries, distance from
	anterior end to center of median oesophageal bulb equal to, or greater
	than distance from center of bulb to base of oesophagus
	· · · · · · · · · · · · · · · · · · ·
	Tail tip usually pointed, often curved ventrally (Fig. 19E). Distance from
	anterior end to middle of median oesophageal bulb less than distance
	from this point to base of oesophagus. One ovary Tylenchus
12.	Vulva at 75% or more of body length
	Vulva at 60% or less of body length
13.	Body short and stout, length about 6 to 10 times greatest width 14
	Body slender, length more than 20 times greatest width (Fig. 12K,L) 16
14.	Body with prominent retrorse (directed posteriorly) annules, usually longer
	than 0.3 mm.; length 10 or more times greatest width. Found in soil 15
	Body without prominent annules, length 6 to 8 times greatest width. Length
	about 0.3 mm. Found in roots
15.	Annules with prominent spines or scales
	Annules without spines or scales (Fig. 121) Criconemoides
16.	Mature female more than 2 mm., often 3-5 mm. long. Found in galls, in
	leaves, or in inflorescense of grains and grasses Anguina
	Body length less than 2 mm. Found in soil or in roots and tubers; sometimes
	in bulbs, leaves and stems
17.	Stylet longer than 3 times width of lip region (Fig. 18C)
	Stylet shorter or about twice width of lip region (Fig. 18A, E)

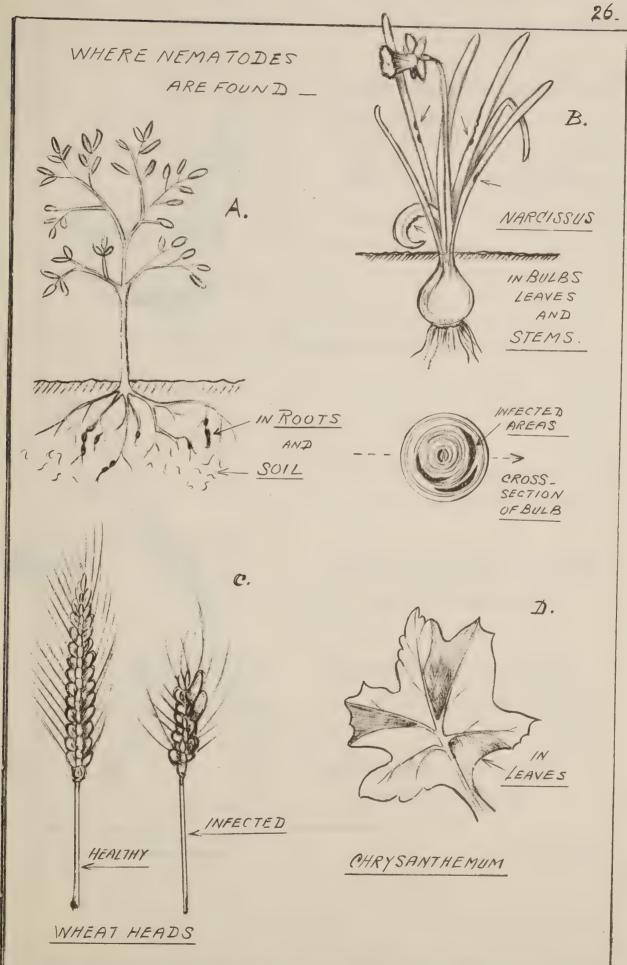
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distance from center of bulb to base of cesophegus
11 tip wantly p curved ventrally (Fig. 19E). Distance from
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Valva ab 60% or less of body length 23
Body short and stout, length about 6 to 10 times greatest width lh
Box slender, length nore than 20 times greatest width (Fig. 12K, E) 16
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than 0.3 mm.; length 10 or more times greatest width. Found in soil 15
Body without prominent annules, length 6 to 8 times greatest width. Length
showt 0.3 am. Found in roots Caupaurus
Annal with prominent spines or scales Criconers
" thout epines or scales (Fig. 12I)
there tends tore than ma., ofte 3-5 mm. long. Found in galls in
or in inflorescense of grains and grasses
then 2 mm. Found in soil or in roots and tubers; sometimes
The access of the send stems of the send o
Sty: Longer than ; times width of lig region (Fig. 18G) 18
encrear or about twice width of hip region (Fig. 18A,E) 19

18.	Posterior portion of body strongly curved ventrally Paratylenchus
	Body usually nearly straight, usually covered by loose cuticle of fourth
	molt
19.	Stylet without knobs or with very small knobs; median oesophageal bulb
	occupying nearly full width of body cavity (Fig. 16C, 18E) 20
	Stylet with distinct knobs; median oesophageal bulb occupying less than
	2/3 of width of body cavity (Fig. 16A)
20.	Tail pointed (Fig. 19G)
	Tail rounded (Fig. 19B)
21.	Tail conical, pointed (Fig. 19G) body long and slender; length 40 or more
	times greatest width. Endoparasitic in bulbs, stems, leaves and tubers,
	from mushroom compost, or sometimes in soil Ditylenchus
	Tail tip rounded
22.	Lip region distinctly set off from body; oesophageal glands forming a
	lobe overlapping intestine; knobs of stylet closely joined. A common
	genus endoparasitic in roots and tubers, also found in soil (Fig. 18A)
	· · · · · · · · · · · · · · · · · · ·
	Lip region not distinctly set off from body; oesophageal gland forming a
	distinct basal bulb (Fig. 16A) knobs of stylet separated like an inverted
	Y. A very rare genus from soil Chitinotylenchus
23.	At least 2 mm. long, slim; length 45 or more times greatest width. Stylet
	very long, six or more times as long as width of lip region (Fig. 18D) 24
	Less than 1.5 mm. long, length of stylet not more than 5 times width of
	lip region
24.	Tail pointed, (Fig. 18C) oesophagus with distinct basal bulb Dolichodorus
	Tail rounded, base of oesophagus a lobe overlapping intestine

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29
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genus endoperse tic in roots and bubers, also found in soil (Fig. 18a)
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constraint a superiques to each list
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25.	Lip region flattened, stylet length about twice width of lip region	
	· · · · · · · · · · · · · · · · · · ·	
	Lip region convex conoid, stylet length 3 or more times width of lip	
	region	26
26.	Tail 2 or more times as long as anal body diameter, tapering (Fig. 19G)	27
	Tail shorter than anal body diameter, rounded (Fig. 19A)	28
27.	Tail tip rounded	nchus
	Tail tip nearly pointed Tetylenchus	
28.	Lip region distinctly set off from body, divided into minute plates.	
	Body at rest or fixed lying only slightly curved Hoplolaimus	
	Annulated lip region continuous with body contour, body usually lies	
	in loose spiral when fixed or at rest. (Fig. 12H)	29
29.	Opening of dorsal oesophageal gland about one-third stylet length or more	
	posterior to stylet knobs Helicotylen	chus
	Opening of dorsal oesophageal gland much less than one-third stylet length	
	posterior to stylet knobs Rotylenchus	

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Carloday ball o o o o o o o o o o o o o o o o o o	
all to Wine the stylet length 3 or nove times width of lip	
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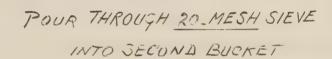
A.

E.

9 19 Lux



PLACE SOIL SAMPLE IN BUCKET,
ADD WATER AND STIR
WITH STICK.
SAND SETTLES TO BOTTOM
NEMATODES REMAIN
SUSPENDED

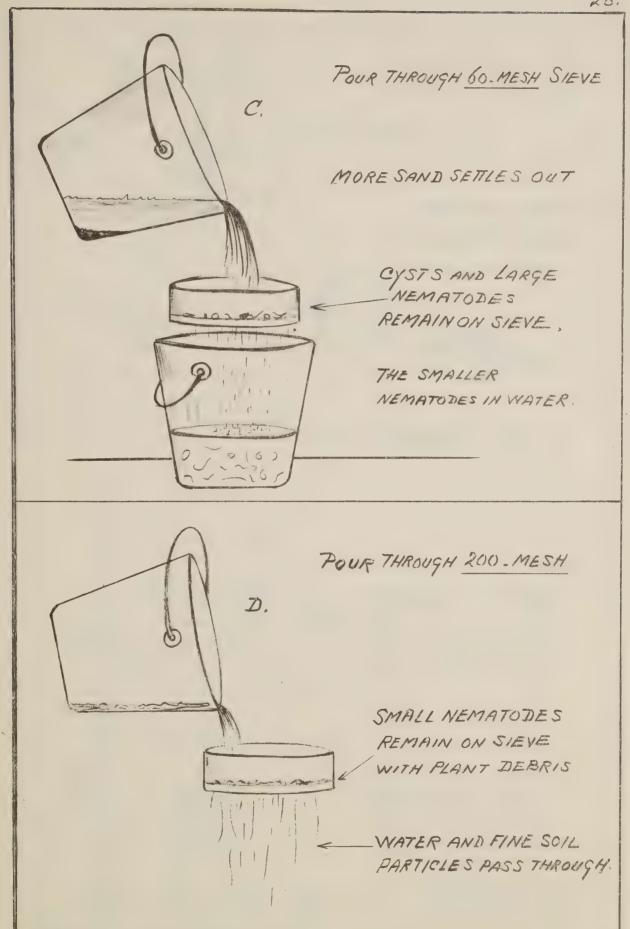


SAND AND GRAVEL REMAIN
IN FIRST BUCKET

SIEVE REMOVES LARGE PIECES OF DEBRIS

NEMATODES PASS THROUGH SIEVE







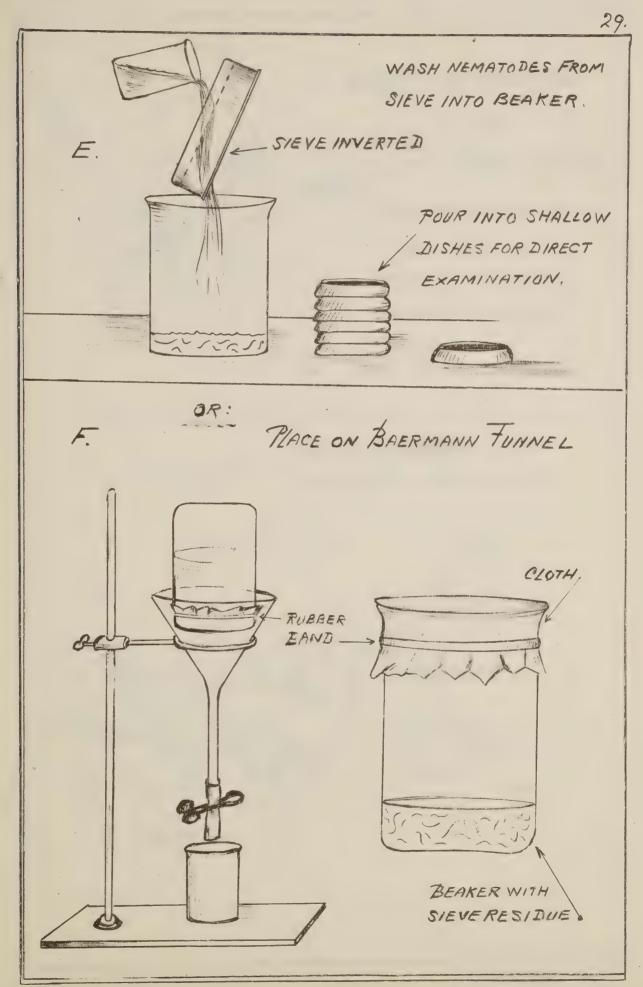
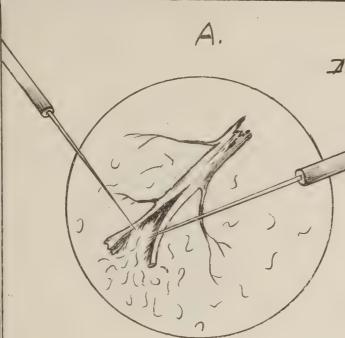


FIGURE - 4.

The second decision of the second sec

BEARER WITH SIEVE PLEBLOGE

a colo for a resi



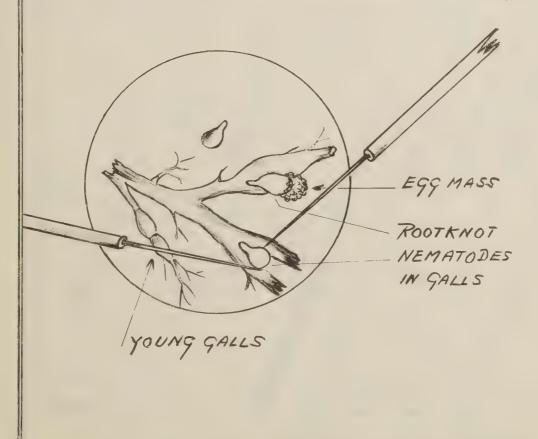
DISSECTING NEMATODES

OUT OF

PLANT TISSUE.

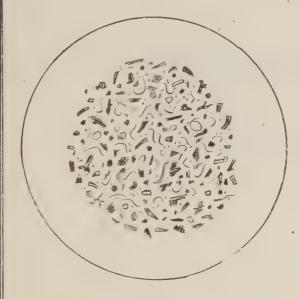
PULL PIECES OF PLANT
TISSUE APART WITH
DISSECTING NEEDLES
UNDER MICROSCOPE.

B. DISSECTING A ROOTKNOT NEMATODE FROM A GALL.



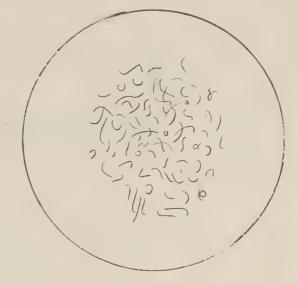


A.

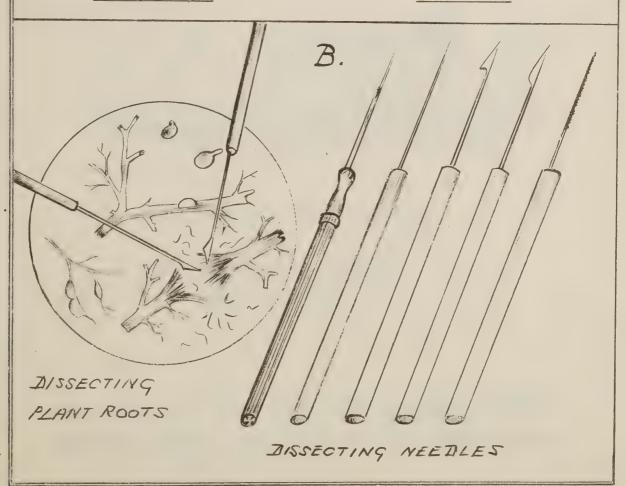


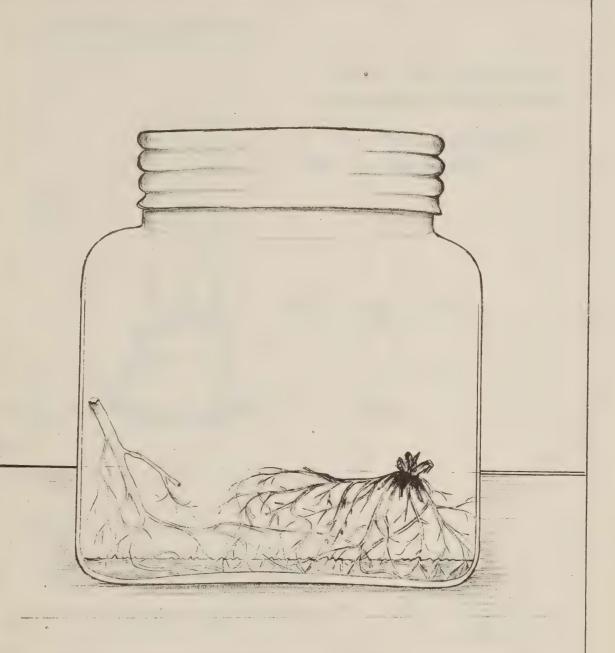
NEMATODES IN DEBRIS.

OBSCURED



NEMATODES FROM BAERMANN FUNNEL CLEAN





INCUBATION TECHNIQUE

FOR

EXTRACTING NEMATODES

FROM ROOTS

WET ROOTS IN FRUIT JAR; ADD A LITTLE WATER; POUR OFF WATER AND EXAMINE FOR NEMATODES,

INTERNATION TECHNIQUE

FOR

ENTRESING NEWATORES

ROOTS ON CRUIT JAKE, AND PLANTE WASTERS

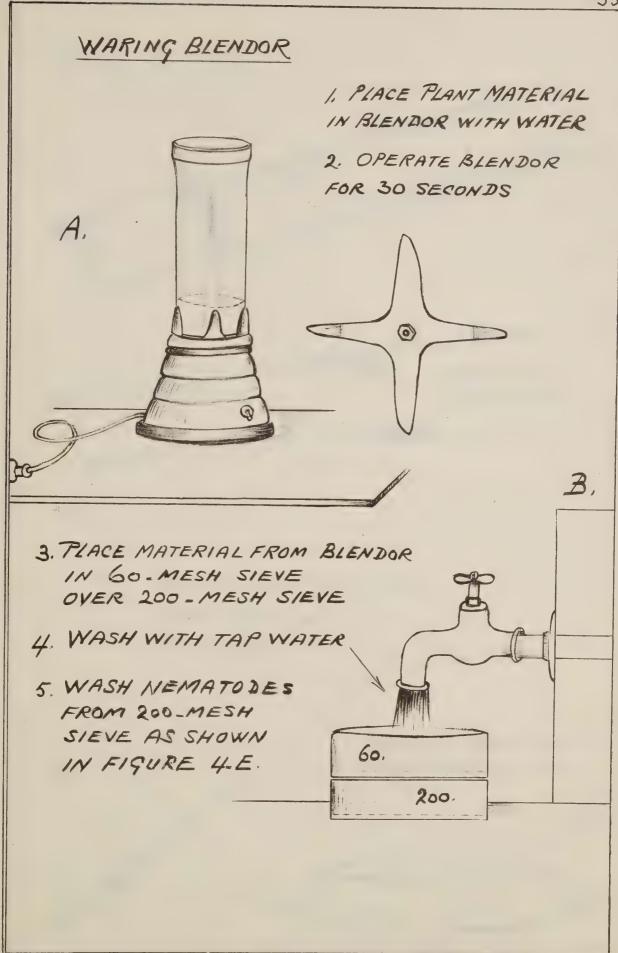


FIGURE - 8.

IN ETENDOR WITH HOSESSAFE

OPERATE RIENDOR

PRINCE MATERIAL FROM BLENDAR IN GO. WESH SIEVE ONER 200. MESH SIEVE

WASH WITH TAP WATER

5. WASH NEMBTODES
FROM 200 MESH
SIEVE AS SHOWN

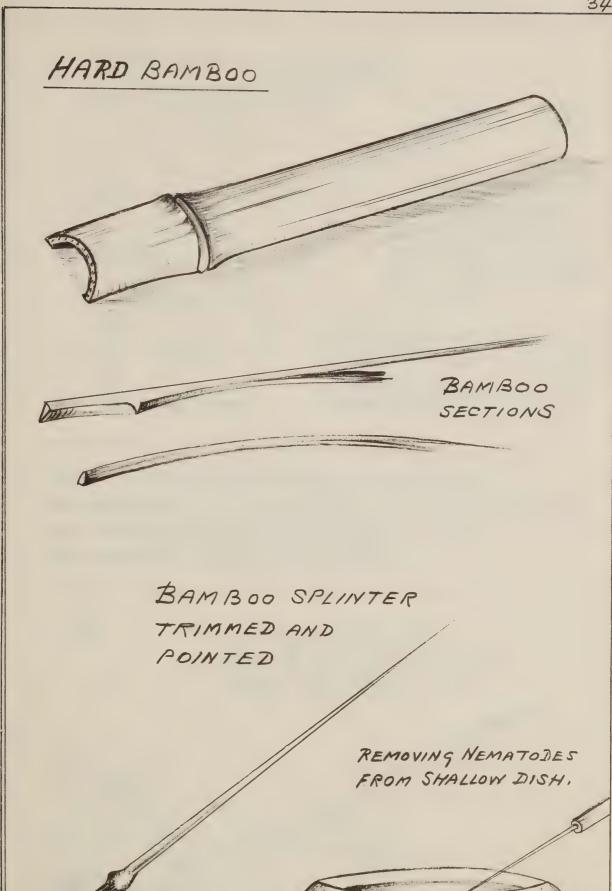


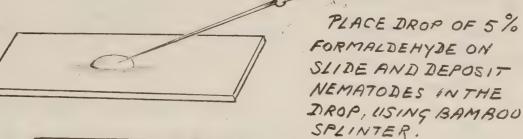
FIGURE - 9.

BARRED AND

REPORTS NENDATODE .

MOUNTING NEMATODES

A.



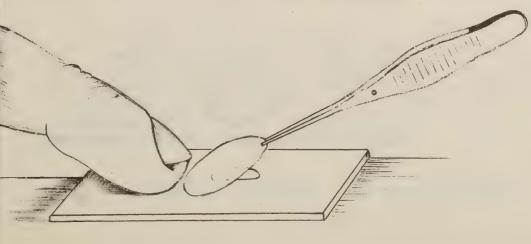


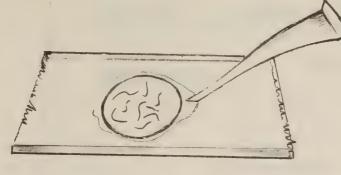
YOU CAN MOUNT AS MANY AS 20 NEMATODES ON ONE SLIDE. PIECES OF GLASS ROD TO SUPPORT COVER GLASS [IF DESIRED]

LOOK AT THE DROP UNDER THE DISSECTING MICROSCOPE AND BESURE THAT THE NEMATODES LIE IN THE BOTTOM OF THE DROP AND IN CONTACT WITH THE SLIDE.

B.

USING FORCEPS, PLACE A COVER GLASS OVER THE DROP.



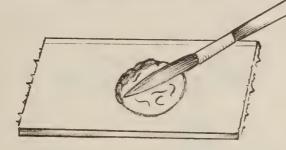


USE A SMALL
PIECE OF FILTER
PAPER, BLOTTING
PAPER OR PAPER
TOWEL TO ABSORB
EXCESS WATER

DOTHIS UNDER THE

DISSECTING MICROSCOPE TO BE

SURE THAT NO NEMATODES ARE LOST.



USING A SMALL B.

BRUSH APPLY A RING

OF HOT PARAFFIN.

VASELINE MIXTURE,

ZUT, OR OTHER

SEALING MATERIAL,

C.



LABEL SLIDE, USING FINE PEN.

SHAPES OF VARIOUS HEMATORES

817971/60A1918

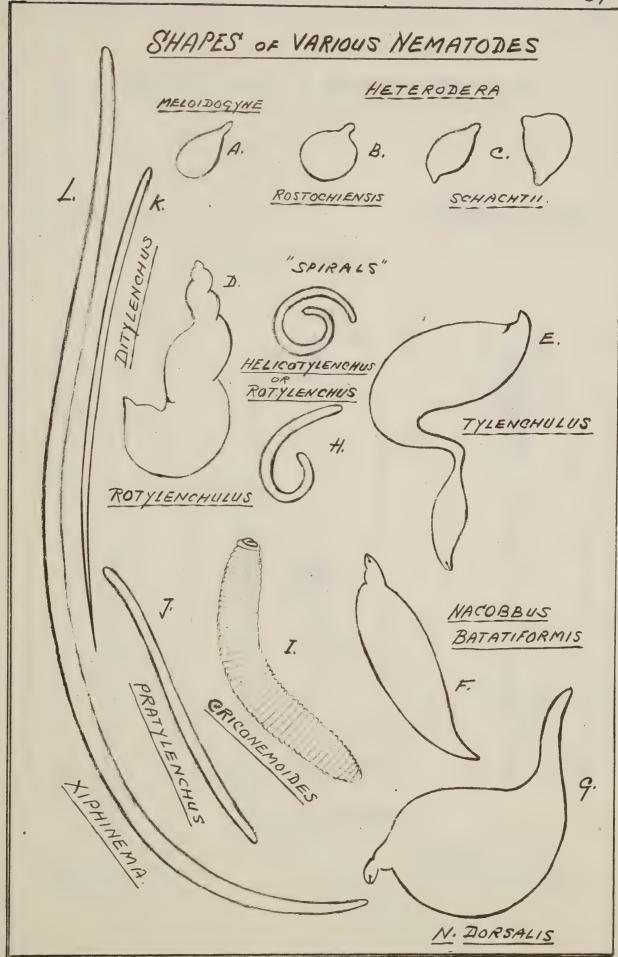


FIGURE 12.

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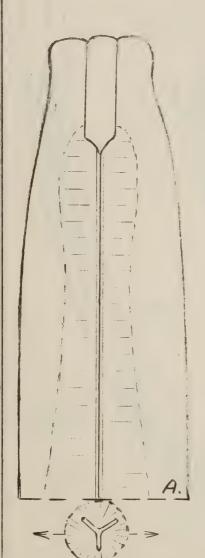
The state of

VARIATIONS IN BUCCAL CAPSULES.

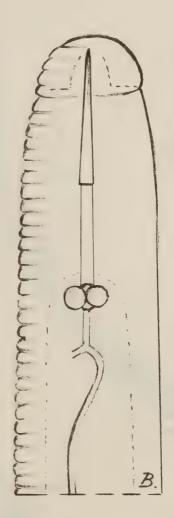
TUBULAR

GDONTOSTYLET.

STOMATOSTYLET.



RHABDITIS



ROTYLENCHUS



DORYLAIMUS

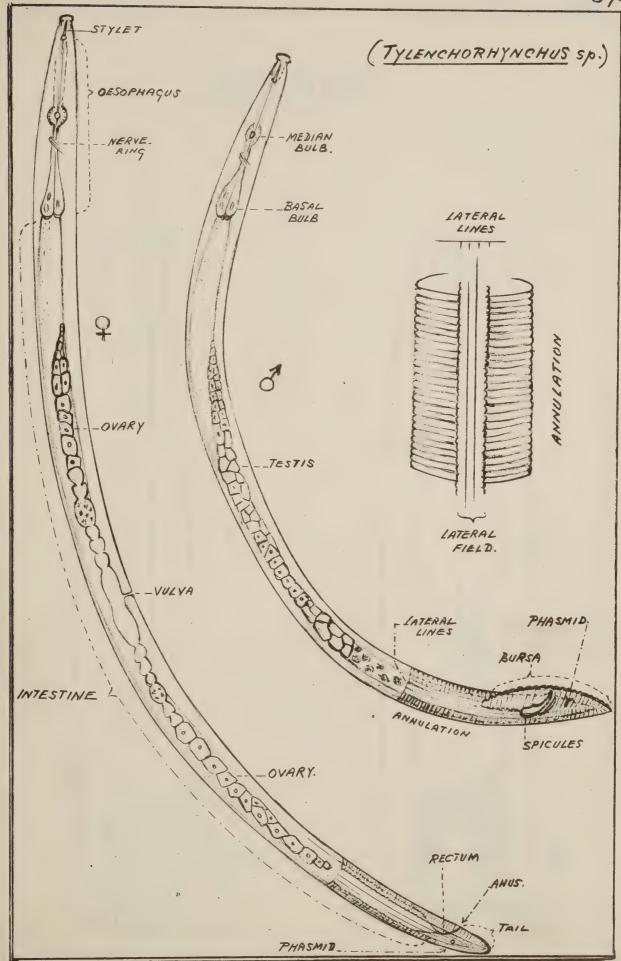
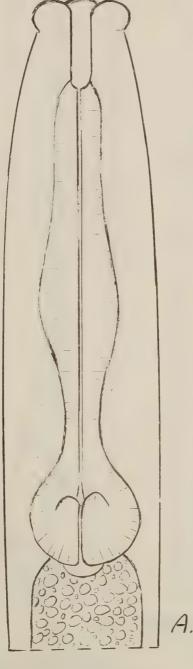
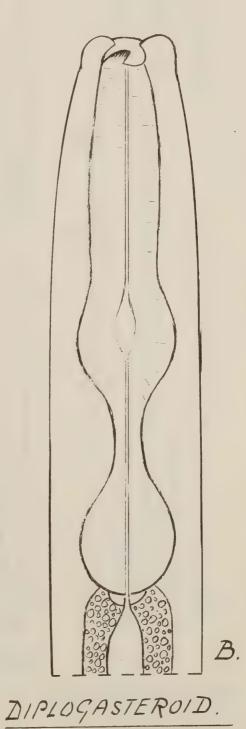


FIGURE 14.

PHASMIDIA.

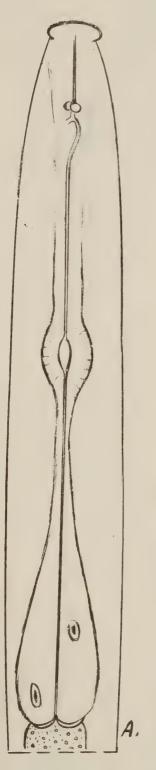


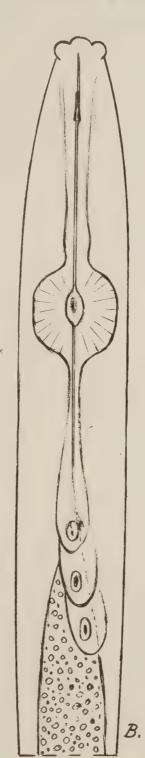
RHABDITOID

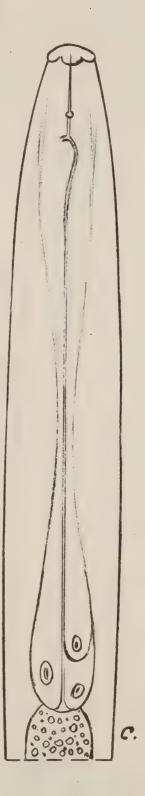




PHASMIDIA







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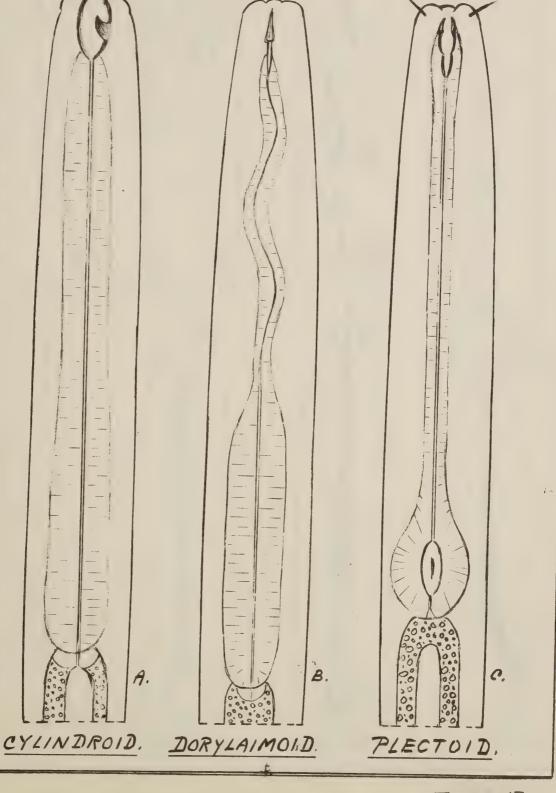
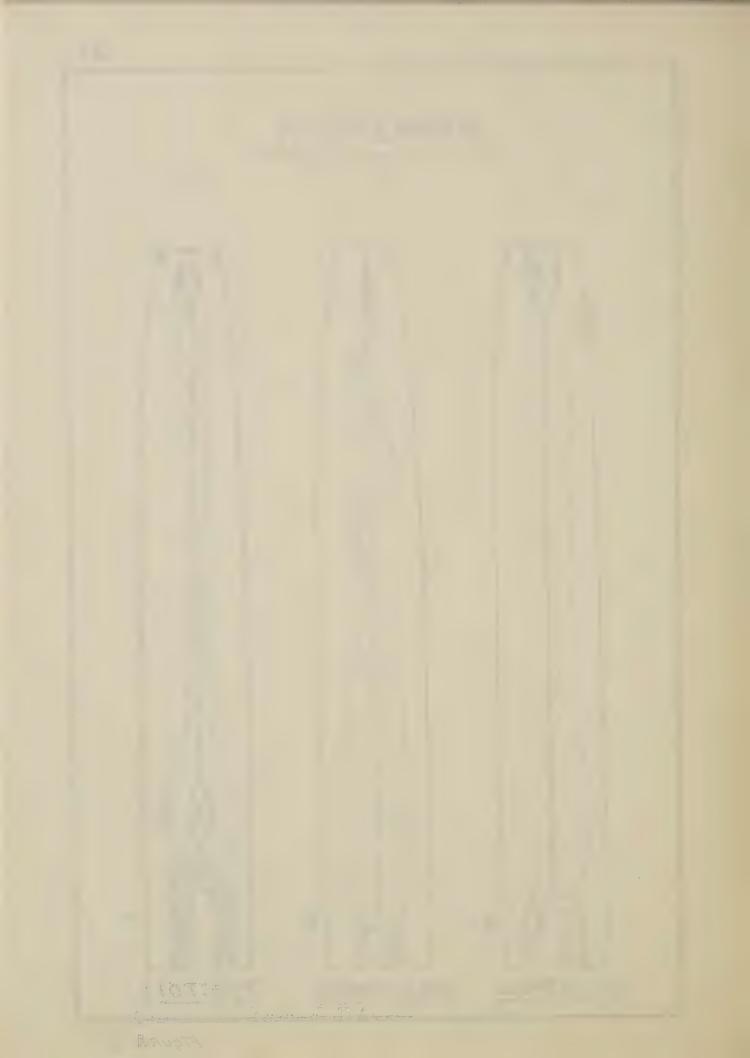


FIGURE 17.



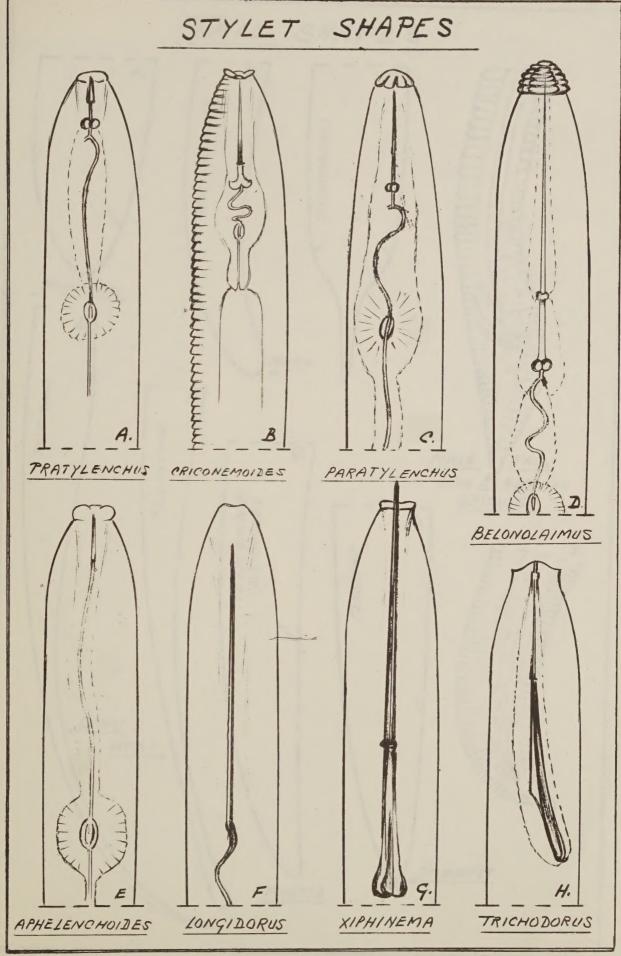
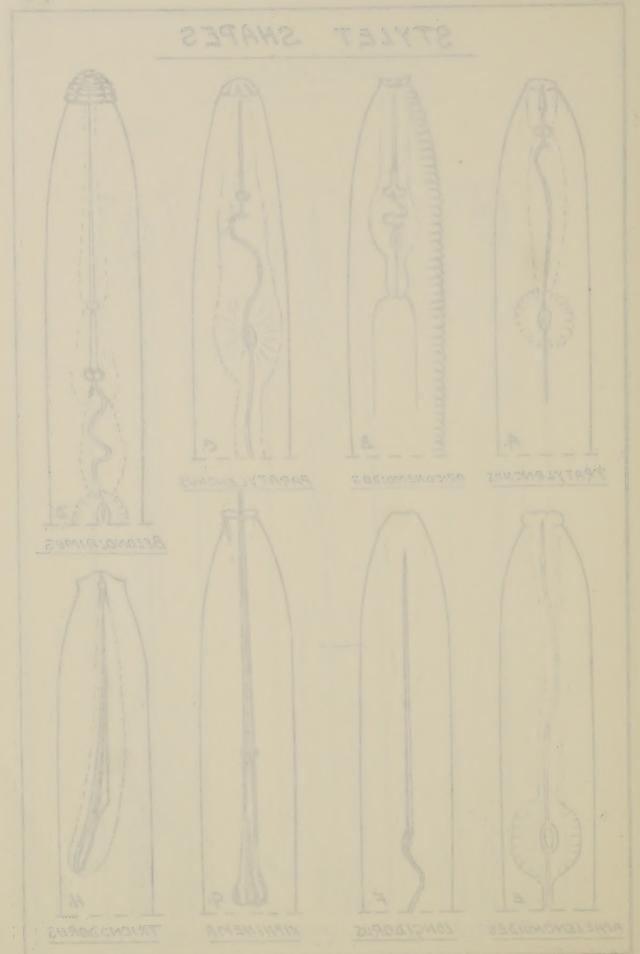


FIGURE 18.



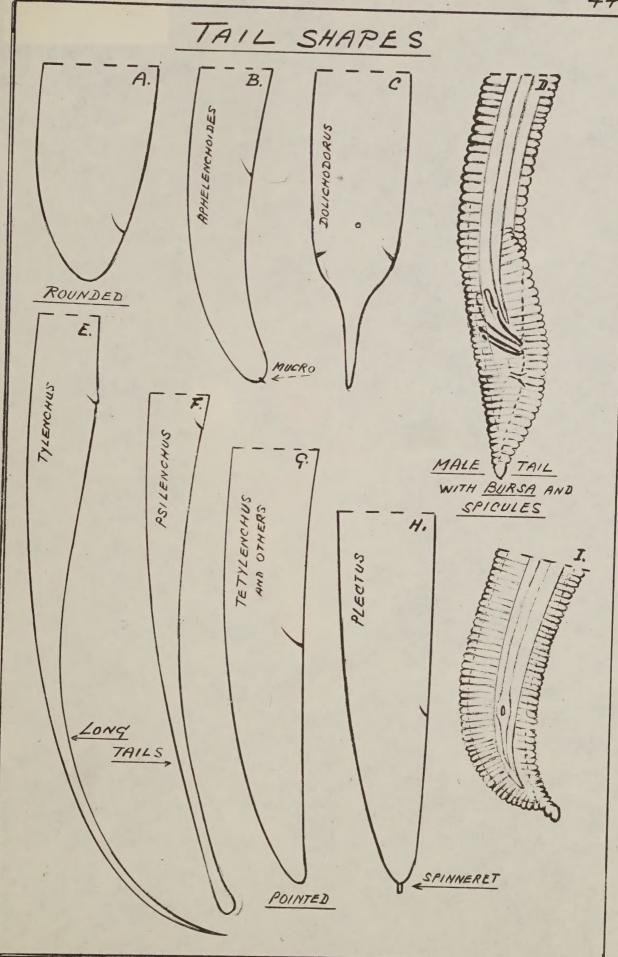


FIGURE 19.

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FIGURE 19.